LA-UR- 01-4493

Automating High-Throughput Finishing at LANL: Process Design, Automation, and Organization

David Bruce, Mark Mundt, Levy Ulanovsky, Heather Blumer, Judy Buckingham, Connie Campbell, Mary Campbell, Olga Chertkov, Joe Fawcett, Tina Leyba, Kim McMurry, Linda Meincke, Chris Munk, Beverly Parson-Quintana, Donna Robinson, Liz Saunders, Judy Tesmer, Sue Thompson, Patti Wills, Norman Doggett, and Larry Deaven

DOE Joint Genome Institute and Center for Human Genome Studies, Los Alamos National Laboratory, Los Alamos, NM 87545

The challenge of high-throughput finishing is being addressed at LANL by integrating sequencing strategies, information management system, automation and personnel organization. The personnel are organized into specialized teams; Informatics, Subclone Re-Array, Template Preparation, Template Labeling, Oligonucleotide Synthesis, DENS, Sequencing, Subcloning, End Sequencing, and Gap Closure. Specialized teams have proven to be a more productive finishing process configuration relative to the "one person - one project" configuration. All samples are handled in a 96 or 384 well format. Library re-array is done using Genetix Q-Bot or Packard MultiProbe robots. Template purification using a solid-phase reversible immobilization (SPRI) method features Robbins Hydra and TiterTek MultiDrop automations. Thermal cycling is done in 384 well format using MJ Research Tetrad. Primer synthesis is done in 96 well format using a Mermaid oligonucleotide synthesizer or differential extension with nucleotide subsets (DENS). Labeling reactions include Big Dye terminator, Big Dye primer, and Big Dye dGTP terminator chemistries. Labeled template is run on capillary ABI PRISM(r) 3700 DNA Analyzers in a 384 well format. The teams are coordinated by instructions generated by an information management system (see LANL finishing abstract, Mundt et al.).

Supported by the US DOE, OBER under contract W-7405-ENG-36.

Map too Large to film

MAP(S) TOO LARGE TO FILM

01 - 4493

Process Design, Automation, and Organization

LASON

2720 C&D Broadbent Pkwy NE Albuquerque, NM 87107

Automating High-Throughput Finishing at LANL: Process Design, Automation, and Organization

David Bruce, Mark Mundt, Levy Ulanovsky, Heather Blumer, Judy Buckingham, Connie Campbell, Mary Campbell, Olga Chertkov, Joe Fawcett, Tina Leyba, Kim McMurry, Linda Meincke, Chris Munk, Beverly Parson-Quintana, Donna Robinson, Liz Saunders, Judy Tesmer, Sue Thompson, Patti Wills, Norman Doggett, and Larry Deaven. DOE Joint Genome Institute and Center for Human Genome Studies, Los Alamos National Laboratory, Los Alamos, NM 87545

Abstract

The challenge of high-throughput finishing is being addressed at LANL by integrating sequencing strategies, information management system, automation and personnel organization. The personnel are organized into specialized teams; Informatics, Subclone Re-Array, Template Preparation, Template Labeling, Oligonucleotide Synthesis, DENS, Sequencing, Subcloning, End Sequencing, and Gap Closure. Specialized teams have proven to be a more productive finishing process configuration relative to the "one person - one project" configuration. All samples are handled in a 96 or 384 well format. Library re-array is done using Genetix Q-Bot or Packard MultiProbe robots. Template purification using a solid-phase reversible immobilization (SPRI) method features Robbins Hydra and TiterTek MultiDrop automations. Thermal cycling is done in 384 well format using MJ Research Tetrad. Primer synthesis is done in 96 well format using a Mermaid oligonucleotide synthesizer or differential extension with nucleotide subsets (DENS). Labeling reactions include Big Dye terminator, Big Dye primer, and Big Dye dGTP terminator chemistries. Labeled template is run on capillary ABI PRISM(r) 3700 DNA Analyzers in a 384 well format. The teams are coordinated by instructions generated by an information management system (see LANL finishing abstract, Mundt et al.).

Template Re-Array

Template Re-Array is the critical to maintaining the flow of the finishing process. 384 well microtiter plates containing all subclones for each BAC project are shipped to LANL from the JGI's Production Genomics Facility in Walnut Creek, CA. These are stored in easily retrievable freezer racks. Computer generated re-array lists are generated for each project to retrieve 2 subclones spanning each finishing target. Technicians retrieve the plates and place these on the deck of the Q-Bot for automated and accurate template re-array. A custom program for the Q-Bot creates skips for controls, duplicate picks to permit multiple reactions (destinations) for any subclone and fully flexible positioning of destination wells.

DNA Preps and Reactions

Subclone growth is performed in GeneMachines HiGro orbital shakers (1) and DNA is isolated following a solid-phase reversible immobilization (SPRI) protocol that features high throughput and low sample cost. Plasmid DNA released by alkaline lysis is tightly bound to the surface of carboxyl-coated magnetic particles, extensively washed, and subsequently released back into solution. A TiterTek Multidrop 384 (2) is used to dispense the lysis solution. Addition of magnetic beads, buffers and recovery of DNA is done with Robbins Hydra's (3). Mixing steps are accomplished with a VWR vortexer (4) with a custom microtiter plate platform. Washing of plasmid DNA is performed with a ELx405 Auto Plate Washer (5).

A specially modified Hydra with 48 syringes and and custom plate positioner is used for setting up "half plate" reactions that have the same set of 48 primers dispensed into two sets of plasmid subclones (6). This optimizes the operation of the Q-Bot for selecting two different subclones for sequencing with the same primers for each target. Cycle sequencing is performed in 384 well format with MJ Tetrads (7).

Custom Primers and DENS

Custom primers are synthesized in house on a Mermade DNA synthesizer (1). The Mermade can produce 192 oligonucleotides per day and two units are in use at LANL. Custom primer reactions are used for providing new sequence across sequence gaps. As an alternate to custom primers we are also using differential extension with nucleotide subsets (DENS). DENS finishing reactions are targeted at improving sequence quality over regions of known but low quality sequence. DENS enables primer walking without custom primer synthesis. The DENS octomer library is accessed with a modified Packard robot (2) with custom plate chillers and a humidified tent to eliminate evaporation of primers.

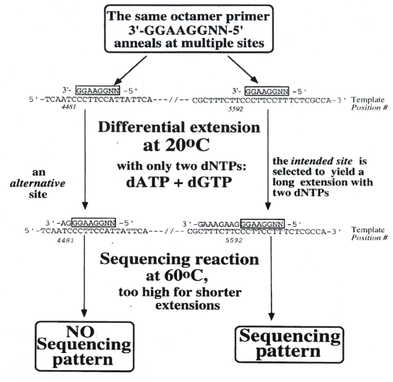
DENS Octomer design

NNAXXXXX
NNCXXXXX
NNGXXXXX
NNTXXXXX

} Library of 1440 octomers:

- **3 to 6 non-degenerate GCs per octomer**
- **Occurrence in human genome ranges from 1 per 1.2 kb to 1 per 45 kb**
- **Library size is 1440 octomers (15 microtiter plates) selected from 4096 possible (hexamer specificity)**
- **Octomers in library are arranged according to their occurrence in genome to maximize efficiency.**

DENS mechanism



Mechanism of DENS. Differential extension of an 8-mer primer (3'-GGAAGGNN-5') using a two-dNTP subset (dATP and dGTP) is shown at two sites: the selected site (by 8 bases) on the right and an alternative site (by 2 bases) on the left. At the subsequent 60°C extension with all four dNTPs, the primer is further extended at the selected, but not alternative site, where it is too short to anneal.